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(54) Abstract Title
Models for neurodegenerative diseases

(57) A recombinant nucleic acid coding for a protein or peptide involved in a neurodegenerative disorder is modified to enable said protein or peptide to be directed to a given cell compartment. The disorder may particularly be Alzheimer's disease, CJD or Huntington's chorea and the recombinant nucleic acid may be obtained by modification of the cDNA encoding for the beta amyloid (1-42) peptide, the prion protein or the huntingtin protein. Cells, or animals comprising such cells, comprising the recombinant molecule or vector therefor may be used to identify molecules which interfere with the development of such disorders or to screen for compounds able to inhibit aggregation of the proteins or peptides produced.

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The present invention relates to the fields of biotechnology, medicine, biology and biochemistry. Its applications relate to the fields of human and animal health. More particularly, the invention relates to methods for producing cell and animal models for neurodegenerative diseases, and to the means (such as

5   nucleic acids or vectors) for implementing such methods.

Neurodegenerative diseases constitute a major and increasing problem to public health, principally linked to a lengthening of the average life span in industrialised countries. Thus Alzheimer's dementia and Parkinson's disease have a real economic impact, not only because of their fatal results but also because of

10   the constraints of long term care imposed by them. While the clinical symptoms have been described and several susceptibility genes have been identified, enabling significant steps to be made in the knowledge of these disorders, the molecular bases supporting the development of these diseases are still very obscure. The elucidation of signalling cascades, which are deregulated in these

15   pathological states, will most probably lead to the discovery of targets suitable for therapeutic intervention. These neurodegenerative diseases are very definitely characterised in part by a poor balance between cell death inducing factors and survival factors. In this regard, the literature is rich in examples which emphasise the importance of alternative splicing in the generation of proteins acting as

20   antagonists on such processes (Bcl2 family, caspases, Grb2, etc...). Any innovation in these fields will have an enormous impact as an effective treatment currently does not exist.

The nature of neurodegenerative diseases renders their study difficult, in particular since an analysis of genetic expression in affected tissue and in healthy controls is almost impossible because of the difficulty of obtaining biopsies of sufficient quantity and quality. Clearly, then, the production of animal and cell

5   models represents a clear advance in understanding the physiopathology of such disorders of the nervous system.

Such models can:

- identify novel cDNA involved in the different stages of the development of neurodegenerative diseases;

10   - test the impact of such cDNA or any construction derived from such cDNA on the starting animal or cell model.

However, no animal model can mirror the whole of a human disorder. There is thus a genuine interest in providing a plurality of models for the same disease, enabling the identifiable genes of interest to be multiplied before

15   evaluating them in a complex human physiopathological situation.

Murine models have been established for a variety of neurodegenerative disorders:

- by transgenesis for Alzheimer's disease (Johnson-Wood et al., 1997, PNAS, 94, pp 1550-1555), amyotrophic lateral sclerosis (ALS) (Gurney et al., 1997, J.

20   Neurol., 244, S15-S20), Huntington's chorea (Davies et al., 1997, Cell, 90, p 537) and prion diseases (Moore, R. C., and Melton, D. W., 1997, Mol. Hum. Reprod., 3, pp 529-544);

- by homologous recombination for Tay-Sachs and Sandhoff diseases (Sango, K. et al., 1996, Nat. Genet., 14, pp 348-352);

- by producing mutant mice for metabolic routes in the case of a model for infantile ceroid lipofuscinosis (Vance et al., Biochim. Biophys. Acta, 1997, 1344, pp 286-299).

Such models reproduce, to a greater or less accurate extent, the behavioural and histological symptoms which are characteristic of the disorders.

Further, such models aim to reproduce the disorders initiated by dominant mutations modelled on those observed in patients but do not provide any information as regards sporadic cases which are not associated with any mutation of the identified susceptible genes and which do not have a hereditary component.

The present application can overcome the disadvantages of the prior art. The present invention describes novel methods and means for producing cell and animal models for neurodegenerative diseases.

The present invention results in part from the observation that the neurodegenerative diseases cited above are all associated with abnormal accumulations of proteins or peptides in precise cell compartments or with accumulations of proteoglycans in pathological cell ultrastructures (Lewy bodies).

In Alzheimer's disease, the accumulated peptides are in the aggregated form. This is the beta amyloid (1-42) peptide from proteolytic cleavage of the protein APP (amyloid precursor protein). Although this peptide is present in the cerebral intercellular space, aggregated into an amyloid sheet, it is produced after specific cleavage of APP in the endoplasmic reticulum where the conditions for its

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aggregation exist (Hartmann, T. et al., 1997, Nat. Med., pp 1016-1020). Such accumulation in the intracellular compartment most probably stresses the endoplasmic reticulum which is known to initiate an increase in the concentration of intracytoplasmic calcium, an initiating factor for apoptotic cascades.

5   The prion protein (Prp) is present in an abnormal conformation in cerebral tissues with spongiform encephalitis (BSE, Kuru, Creutzfeld-Jakob disease). This conformation is characterized by a resistance to digestion by proteinase K. The endosomal compartment has been described as being the location where conversion then aggregation of the abnormal form of Prp occurs (Arnold, J. E.,

10   1995, J. Pathol., 176, pp 403-411).

Huntington's chorea is characterized by aggregation of a modified form of a cell protein. This is huntingtin modified by glutamic acid repeats of varying length at the NH_2 terminal end of the protein. The aggregates are detected in dystrophic neurites and in nuclear inclusions, these two locations reflecting a

15   retrograde transport of huntingtin, linked to its function which has not yet been elucidated (DiFiglia, M. et al., 1997, Science, 277, pp 1990-1993).

These three examples illustrate the phenomena of accumulation of proteins or peptides in cell compartments in the development of these disorders. The aim of the present invention is to provide models which can reproduce the cellular

20   deregulations linked to protein aggregation. These models can be animal models obtained by transgenesis, or cell models. These cell models can be obtained by transfection and can be derived from neuronal cells or from other cell types, such as fibroblasts.

In a first aspect, the invention more particularly provides recombinant nucleic acids coding for proteins or peptides involved in neurodegenerative disorders, modified to enable said proteins or peptides to be directed to a given cell compartment.

5   More particularly, the invention provides recombinant nucleic acids coding for proteins or peptides involved in neurodegenerative disorders, modified to enable said proteins or peptides to be directed to a given cell compartment, with the aim of causing their aggregation or conformational changes.

In a further aspect, the invention provides expression products of the above recombinant nucleic acids, and any vector comprising such a recombinant nucleic acid.

The invention also encompasses any cell comprising a recombinant nucleic acid or vector as defined above. Advantageously, it is a cell which can express said recombinant nucleic acid so as to produce the protein or peptide in a given cell compartment.

Still further, the invention encompasses any animal (non human) with one or more recombinant nucleic acids or vectors as defined above in some or all of its cells.

Finally, the invention claims the use of these cells or animals for the identification of molecules which can interfere with the development of neurodegenerative disorders.

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The possibilities of obtaining models by addressing proteins or peptides in precise cell compartments can be illustrated, for example, for Alzheimer's, Creutzfeld-Jakob and Huntington's disease.

Thus a particular recombinant nucleic acid of the invention is represented by

5 any modification of the cDNA coding for the beta amylogenic (1-42) peptide with the aim of directing this peptide to a given cellular compartment with the aim of causing its aggregation. More particularly, this modification can be constituted by firstly adding, upstream of the sequence coding for the beta (1-42) peptide, a nucleic acid sequence corresponding to a secretion sequence permitting secretion in the

10 endoplasmic reticulum and coding for a succession of hydrophobic amino acids (sequences which are well known to the skilled person) and secondly, downstream addition of the KDEL amino acid concatenation, thus specifying retention of the aggregating form of the amyloid peptide in the endoplasmic reticulum (Griffiths, G. et al., 1994, J. Cell. Biol., 127, pp 1557-1574).

15 A further particular recombinant nucleic acid of the invention is represented by any modification in the cDNA of the prion protein with the aim of directing this protein to a given cell compartment with the aim of provoking its pathological conformation there. More particularly, this modification may be downstream addition to the sequence coding for the Prp protein of the cDNA sequence coding

20 for the cytoplasmic region of the cation-dependent receptor of mannose-6-phosphate (Rohrer, J. et al., 1995, J. Cell. Biol., 130, 1297-1306). This modification must allow localisation of the modified Prp protein in the endosomes to encourage pathological transconformation.

A further particular recombinant nucleic acid of the invention is represented by any modification in the cDNA of the huntingtin protein with the aim of directing this protein to a given cell compartment with the aim of causing its aggregation therein. Precisely, this modification is carried out on the cDNA of

5   huntingtin with a sequence coding for a repeat of 115 to 156 glutamic acids added at the 5' end which endows this protein with aggregating properties (Davies et al., 1997, Cell, 90, p 537). More particularly, this modification can be carried out by inserting a nuclear localisation sequence into this protein, the archetype for which is represented by the amino acid concatenation PKKKRKV (Efthymiadis, A. et al.,

10   1997, 272, pp 22134-22139). Advantageously, this nuclear localisation sequence is added to the C-terminal end of huntingtin, and thus does not interfere with the glutamic acid repeats at the origin of the aggregating properties which are located at the NH$_2$ terminal end of this protein.

The recombinant nucleic acids of the invention may be DNA

15   (complementary, genomic, synthetic, semi-synthetic, etc...) or RNA. Further, these nucleic acids may comprise transcription signals (promoter, terminators, etc...).

The recombinant nucleic acids of the invention can be inserted into any construction of viral vectors (retrovirus, adenovirus, AAV, herpes virus) or non

20   viral vectors permitting expression of one of the modified proteins or peptides described above in cells. Advantageously, it is an adenoviral vector which can infect non proliferative cells such as primary neuron cultures. A further particular vector of the invention is a construction of vectors allowing the production of

transgenic animals expressing one of the modified proteins described above in their neurons.

The invention also encompasses establishing cellular clones constitutively or inducibly expressing one of the modified proteins described above.

5   Advantageously, these clones are derived from cells with certain neuronal differentiation characteristics, such as PC12 cells.

The invention also encompasses the animals described above and any use of such transgenic animals for preparing primary cultures of neurons expressing one of the modified proteins as described above.

10   The invention also encompasses the use of one of the modified proteins as defined above and expressed in acellular, cellular or animal models for screening or identification of compounds or compositions, in particular chemical compositions, with the property of inhibiting aggregation of the proteins in question.

15   The invention further encompasses any use of one of the modified proteins as described above and expressed in cell or animal models with the aim of screening or identification of compounds or compositions, in particular chemical compositions, for their abilities to inhibit apoptosis induced by aggregation of the proteins discussed.

20   The invention still further encompasses methods for screening or identification of compounds with the property of inhibiting aggregation of the proteins involved in neurodegenerative disorders or with the capacity of inhibiting apoptosis induced by aggregation of such proteins. These methods comprise

bringing a cell or animal as defined above into the presence of a test compound or composition and observing the effect on aggregation or apoptosis.

The present invention will now be described in more detail with the aid of the following examples, which are given by way of illustration and are in no way

5   limiting.

1. Example of a model of cell stress induced by an accumulation of amyloid

peptide in the endoplasmic reticulum.

A secretion sequence and a sequence for retention in the endoplasmic reticulum, i.e., the amino acid concatenation KDEL, are added to the beta amyloid

10   (1-42) peptide using conventional molecular biological techniques which are known to the skilled person.

Since the aim of this construction is to express proteins with the ability to aggregate and consequently to induce cell apoptosis, the cDNA sequence coding for the modified amyloid peptide is advantageously introduced into an inducible

15   expression vector. Advantageously, the inducible vector is selected so as to ensure strict repression of the transgene in non induced conditions, in particular in the presence of tetracycline, and to enable strong induction during withdrawal of this antibiotic.

Since the aim of this model is to identify strategies or chemical

20   compositions to inhibit aggregation of the beta-amyloid peptide or to improve cellular viability, the human line HeLa is studied as the cell model in which to establish an inducible expression system for the selected transgene. This non

neuronal line possesses high efficiency of transfection and an absence of basal expression level in the presence of tetracycline.

The HeLa line constitutively expressing the *tet* repressor is commercially available (Clontech).

5 The vector containing the cDNA coding for the modified beta amyloid (1-42) peptide in accordance with the foregoing is introduced by transfection using techniques which are known to the skilled person. HeLa clones which have integrated this genetic construction are selected by means of co-transfection of a second plasmid which causes resistance to the selection agent. Advantageously,

10 the agent selected is hygromycin.

The hygromycin-resistant HeLa clones are selected by characterizing the expression of the modified peptide when inducing expression by tetracycline withdrawal.

Typically, the 5 clones enabling the strongest expression are selected for

15 further study.

Of that five, clone 15.09, which has the best viability in the presence of tetracycline and which has the strongest apoptosis in the absence of this antibiotic (demonstrated by degradation of genomic DNA) is then characterized morphologically.

20 Cell biology and immunohistochemical techniques reveal an accumulation of modified peptide in the endoplasmic reticulum, specifically in the absence of tetracycline. Further, a significant enlargement of this organelle is visible in the hours following de-repression of expression of the modified peptide.

In addition to the genomic DNA degradation motifs which are revealed by electrophoresis, the apoptosis induced in HeLa cells is correlated with a high activation of stress kinases JNK1 and JNK2.

None of these apoptotic markers is observed in clones expressing the beta

5   amyloid peptide fused to an ELKD sequence (no longer KDEL) used as a control, nor are they observed in the starting HeLa line. Similarly, when a peptide of 42 amino acids corresponding to the same amino acids as the beta amyloid peptide but in a different order is fused to the KDEL sequence, no alteration in cellular ultrastructures, no modification in the genomic DNA and no constitutive

10   activation of stress kinases are observed.

These results document the production of an apoptosis model by retention of an aggregating peptide in the endoplasmic reticulum which is most relevant to Alzheimer's disease.

This model has the following applications:

15   • use of a cell model based on the strategy discussed above with the aim of identifying chemical compounds which can inhibit aggregation of the beta-amyloid peptide.

• use of a cell model based on the strategy discussed above with the aim of identifying chemical compounds which can inhibit apoptosis induced by

20   aggregation of the beta amyloid peptide.

10. Use according to claim 8 for screening or identification of compounds or compositions which can inhibit apoptosis induced by aggregation of beta amyloid peptide.

11. A recombinant nucleic acid substantially as hereinbefore described.

12. A protein or peptide substantially as hereinbefore described.

13. A vector substantially as hereinbefore described.

14. A cell substantially as hereinbefore described.

15. Use of a cell or animal in a method for screening or identification purposes substantially as hereinbefore described.

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Claims searched: 1-15

Examiner: L.V.Thomas
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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q):

Int Cl (Ed.6): A01K 67/027; C12N 15/12

Other: Online: EPODOC, WPI, BIOSIS, SCISEARCH

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	WO 95/05466 A1 (INST. OF PSYCHIATRY) see p.4 1.1 - p.6 1.27	
P,A	J. Biol. Chemistry (1998) 273(11) pp.6277-6284 - Tomita et al.	

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